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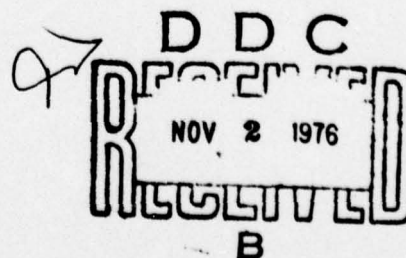
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Cortex of rat kidney was homogenized and fractions enriched in plasma membrane, endoplasmic reticulum or brush border were prepared by several techniques of differential centrifugation. The identity and homogeneity of the membrane fragments were investigated by assaying marker enzymes and by transmission and scanning electron microscopy. Kallikrein was present in both plasma membrane and endoplasmic reticulum enriched fractions isolated by two fractionation procedures. Kallikrein was highly concentrated in plasma mem-

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20. Abstract

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It is suggested that after renal kallikrein is synthesized on endoplasmic reticulum, it is subsequently reoriented to a surface membrane for activation and release. Renal kallikrein may enter the tubular filtrate distal to the proximal tubules. The brush border membrane of proximal tubule is the major site of inactivation of kinins and angiotensin II.

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ISOLATION OF MEMBRANE-BOUND RENAL ENZYMES THAT METABOLIZE KININS AND ANGIOTENSINS

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Synopsis

Cortex of rat kidney was homogenized and fractions enriched in plasma membrane, endoplasmic reticulum or brush border were prepared by several techniques of differential centrifugation. The identity and homogeneity of the membrane fragments were investigated by assaying marker enzymes and by transmission and scanning electron microscopy. Kallikrein was present in both plasma membrane and endoplasmic reticulum enriched fractions isolated by two fractionation procedures. Kallikrein was highly concentrated in a plasma membrane fraction but was absent from the brush border membrane of proximal tubular cells. Cells of transplanted renal tumors of the rat, originating from the proximal tubule, had no kallikrein activity. Kininase activity, angiotensin 1-converting enzyme (kininase II) and angiotensinase were found in a plasma membrane enriched fraction and especially in the fraction containing isolated brush border.

It is suggested that after renal kallikrein is synthesized on endoplasmic reticulum, it is subsequently reoriented to a surface membrane for activation and release. Renal kallikrein may enter the tubular filtrate distal to the proximal tubules. The brush border membrane of proximal tubule is the major site of inactivation of kinins and angiotensin II.

Abbreviations

PM: plasma membrane

ER: endoplasmic reticulum

ATPase: adenosine triphosphatase

SBTI: soybean trypsin inhibitor

It has been known for over half a century that urine contains kallikrein (E.C. 3.4.21.8; Frey et al., 1968). Hypertensive patients excrete less of this hypotensive enzyme than normotensive subjects (Elliot and Nuzum, 1934; Margolius et al., 1974). Although urinary kallikrein is believed to originate from the kidney (Frey et al., 1968), some of the properties of urinary and renal kallikrein differ. For example, there appears to be more activity in the urine of the rat than can be expected from the activity found in the kidney. Carvalho & Diniz (1966) suggested that kallikrein is present in the lysosomal fraction of homogenized kidney, but Nustad (1970) found it in a microsomal fraction. In addition to kallikrein, the kininogenase that releases kinins, urine also contains the vasoactive peptides bradykinin, kallidin and met-lys-bradykinin (Werle & Erdös, 1954; Miwa et al., 1968; 1969).

Kinins are natriuretic, diuretic, hypotensive and they release prostaglandins from the medulla of the kidney (Webster & Gilmore, 1964; Barraclough & Mills, 1965, McGiff et al., 1976). These properties indicate that they may have important effects on some aspects of renal function. The kidney is very rich in kininases that terminate the action of kinins. Most of the kininase activity sediments with the microsomal fraction. Erdös & Yang (1966) described three renal kininases. One of these is similar to kininase I or carboxypeptidase N (E.C. 3.4.12.7) of plasma (Erdös & Sloane, 1962). A second and probably the most important one is kininase II, (E.C. 3.4.14.1) which is identical with the angiotensin I-converting enzyme (Erdös & Yang, 1967; Yang et al., 1970; 1971; Oshima et al., 1974; Erdös, 1976). The ratio of activity of kininase I to kininase II is about 1 to 4 in the renal cortex (Erdös & Yang, 1966; Ward & Mills, 1975).

Because of the importance of angiotensin and bradykinin in renal function, we studied the cellular and subcellular localization of enzymes that metabolize them. We isolated fractions highly enriched in plasma membrane (PM), endoplasmic reticulum (ER) or in brush border of renal proximal tubules. The identity of the fractions was confirmed by both transmission and scanning electron microscopy and by assaying marker enzymes. We found that the brush-border membrane of the proximal tubules contains kininase, angiotensin I-converting enzyme and angiotensinase, but virtually no kallikrein. Kallikrein is concentrated in a PM and also in an ER-enriched fraction. Transplanted renal tumors, which originated from the cells of the pars recta of proximal tubules, contained no kallikrein activity. Some of these experiments were briefly summarized in an abstract (Ward *et al.*, 1975a) and in a short communication (Ward *et al.*, 1975b).

Methods

Animals. Unanesthetized male Sprague-Dawley rats weighing 250 to 350 g were killed by decapitation or cervical dislocation. The aorta was clamped below the renal arteries, the vena cava opened, and the kidneys perfused through the aorta using a solution of 0.25 M sucrose and 5 mM-$NaHCO_3$, pH 7.5 or 0.9% NaCl until they were free of blood. In control studies it was ascertained that starvation for 24 h before sacrifice made no difference in the results. All subsequent procedures were carried out at $4^{\circ}C$. For each experiment, about 10 g of renal cortex dissected from three to six rats was pooled and minced.

Membrane Fractionation I. Renal tissue was suspended in 9 vols of buffer containing 0.25 M sucrose, 0.2 mM-$MgSO_4$, and 10 mM-Tris-HCl (pH 7.4).

The tissue was disrupted gently with a very loosely fitting Dounce homogenizer. The cells were completely disrupted by nitrogen cavitation (Dowben et al., 1968) after equilibration with nitrogen at 800 lb/in^2 (5.6 MPa) for 20 min in an Artisan pressure homogenizer. EDTA was added to 1 mM concentration and the homogenate centrifuged at 10,000 g for 15 min. The supernatant fraction was then centrifuged at 30,000 g for 15 min. The pellets, containing nuclei, mitochondria, lysosomes, and cell debris, were discarded. Microsomes in the supernatant fraction were harvested by sedimentation at 100,000 g for 60 min in a Sorvall OTD-2 ultracentrifuge. The microsomal pellet was suspended first in 10 mM-Tris (pH 8.6), sedimented at the above speed, resuspended in 1 mM-Tris (pH 8.6) and sedimented again. Finally, the microsomal pellet was suspended in about 30 ml 1 mM-MgSO$_4$ plus 1 mM-Tris (pH 8.6) and dialyzed against the same buffer for 2 h.

Highly enriched fractions of PM and ER were obtained using a modification (Birckbichler et al., 1973) of the method of Wallach & Kamat (1966). The microsomal suspension was carefully layered on 20 ml 15% (w/w) Dextran 110 in 1 mM-MgSO$_4$ and 1 mM-Tris-HCl (pH 8.6) ρ = 1.06 g/cm^3. The discontinuous gradients thus formed were centrifuged in a SW-27 swinging bucket rotor at 27,000 rev/min (r = 11.5 cm) for 15 h. The PM-rich fraction concentrated at the interface and the ER-rich fraction pelleted. Both fractions were collected, washed once in 10 mM-MgSO$_4$ and 10 mM-Tris (pH 8.6), and then suspended in 5 mM-Tris (pH 8.6).

Membrane Fractionation II. Washed microsomes from renal cortex were prepared and separated into ER and PM components according to a procedure developed specifically for rat kidney cortex (Jakobsson, 1974). Renal cortices were homogenized in 20% (w/v) 3 mM-Tris (pH 7.5) with six to

eight strokes of a glass-teflon homogenizer (Thomas, size B) at 700 rev/min. The sucrose concentration was then brought to 0.25 M and the homogenate was centifuged at 10,000 g for 20 min to remove cell debris, nuclei, mito-chondria and lysosomes. Approximately 8 ml of the supernatant was layered on a 3.5 ml cushion of 1.6 M-sucrose and centrifuged at 105,000 g for 30 min with a SW 27.1 rotor in a Beckman L5-65 ultracentrifuge. The micro-somes at the cushion interface were removed, combined ($\approx$ 9 ml) and diluted to approximately 15 ml.

Continuous linear gradients were prepared with Ficoll increasing in concentration from 0 to 8% (w/v) in 10% (w/v) sucrose. Ficoll was previously purified by dialysis against doubly distilled water for 48 h at 4°C. Approximately 6 ml of the microsome concentrate was then applied to the top of each of two 25 ml gradients and centrifuged in a SW 25.1 swinging bucket rotor at 50,000 g for 45 to 60 min. After centrifugation, 2 ml fractions were pipetted sequentially from the top of each gradient, diluted with 20 ml of 10 mM-Tris (pH 8.6) and centrifuged at 100,000 g for 45 min. The pellets were suspended in 1 mM-Tris (pH 8.6), centrifuged for 45 min and resuspended in appropriate buffer. All samples were assayed within 48 h.

Brush Border Preparation. Large fragments of brush-border membranes from the proximal convoluted tubular cells were prepared by the method of Wilfong & Neville (1970).

Minced cortex was homogenized in 100 ml of 20 mM-NaHCO$_3$ (pH 8.1) with a hand held loose glass-teflon homogenizer (6 strokes). Two hundred and twenty ml of 10 mM-NaHCO$_3$ (pH 8.1) was added and the homogenate was stirred for five minutes. The homogenate was poured through coarse and

subsequently fine nylon screen ($\simeq$ 150 micron openings). The filtrate was centrifuged at 500 g for 20 min and the pellet collected. This procedure was repeated a second time. The pellets were resuspended in approximately 28 ml of 10 mM-NaHCO$_3$ (pH 8.1) and 60 ml of 60% (w/w) sucrose was added. The final concentration of sucrose was adjusted to 40.9 $\pm$ 0.1% (w/w) and 1 ml of 40.4 $\pm$ 0.1% sucrose was layered over the solution. After centrifugation at 90,000 g for 75 min, the brown floating layer was removed and pelleted in 4 mM-NaHCO$_3$ (pH 8.1) and 1 mM-MgCl$_2$ at 3,000 g for 10 min.

A linear gradient of 3 to 21% (w/w) sucrose in 4 mM-NaHCO$_3$ (pH 8.1) and 1 mM-MgCl$_2$ was formed over a 1.5 ml cushion of 41 $\pm$ 0.1% sucrose. The resuspended pellet was layered on the gradient and centrifuged at 2,500 g for 15 min. The brush-border fraction, which collected at the gradient-cushion interface, was diluted to 10 ml in 4 mM-NaHCO$_2$ (pH 8.1) and 1 mM-MgCl$_2$ and pelleted at 20,000 g for 20 min. This brush-border fraction was then assayed and embedded for transmission and scanning electron microscopy.

Tumors. Frozen Morris renal tumors MK2 and MK3, together with host animal kidneys and control kidneys, were obtained from Dr. George Weber of the Department of Pharmacology, Indiana University School of Medicine, Indianapolis. Transplanted Morris tumors of rat adenocarcinomas are probably derived from proximal renal tubules of the rat (Hruban et al., 1973). Tumors, host kidneys and normal kidneys were thawed, weighed and suspended in 10% (w/v) 20 mM-Tris (pH 7.5) with an all glass Potter-Elvehjem homogenizer or a Polytron homogenizer. The homogenate was solubilized with Triton X-100 at a final concentration of 0.1%. After 15 min at 4°C and centrifugation at 10,000 g for 5 min, the supernatant was dialyzed against the same buffer for approximately 2 h.

Enzyme Assays. Renal kallikrein was assayed by incubating fractions of homogenized renal tissue (50–100 µl) with kininogen substrate (100 µl) in 0.1 M-Tris, pH 8.5 (500 µl), in the presence of the kininase inhibitors, nonapeptide SQ 20881 (100 µl, 1 mM), EDTA (100 µl, 50 mM) and o-phenanthroline (100 µl, 10 mM) at 37°C (Erdös, 1971; Yang et al., 1971). The amount of kinin generated was determined on an isolated rat uterus (1 unit of kallikrein activity is the amount of enzyme that forms the equivalent of 1 ng of bradykinin in 1 min). Incubation of synthetic bradykinin with renal extracts showed that kininases were completely inhibited. Kininogen was prepared by heating dog plasma at 61°C for 30 min. The kininogen preparation had no residual kallikrein or kininase activity when incubated at 37°C. In inhibitor studies, aprotinin (12 µg/ml) and SBTI (100 µg/ml) were preincubated for 15 min at 37°C before the kininogen substrate was added. In control experiments, we ascertained that the peptide released from dog plasma substrate was not angiotensin. Using specific inhibitors of kallikrein and differential bioassay on the isolated rat uterus and duodenum, we confirmed that all the material released from plasma that contracted the rat uterus was a kinin. The esterolytic activity of kallikrein was assayed with α-N-tosyl-L-arginine(^{3}H)methylester substrate (Beaven et al., 1971).

Renal kininase and angiotensinase were assayed by incubating samples of renal homogenate (20 µl), with bradykinin or angiotensin II (200 ng in 200 µl) in 0.1 M-Tris, pH 7.4, 0.2 M-NaCl (200 µl) at 37°C then following the inactivation of the peptide on the isolated rat uterus (1 unit of kininase or angiotensinase activity is the amount of enzyme that inactivates

the equivalent of 1 μg of bradykinin or angiotensin II in one min under the described conditions).

Renal angiotensin I-converting enzyme (kininase II) was assayed by incubating renal fractions (50 μl), with 1 mM hippurylglycylglycine in 0.2 M-Tris, pH 7.4 containing 0.2 M-NaCl at 37°C (Yang et al., 1971). Converting enzyme activity was calculated as the amount of hippurylglycyl-glycine hydrolyzed that could be inhibited by 0.1 mM of the specific inhibitor, SQ 20881. The amount of diglycine released was assayed in a Beckman 121C amino acid analyzer.

Membrane fractions were characterized by several marker enzymes. Alkaline phosphatase (E.C. 3.1.3.1) was assayed with p-nitrophenyl phosphate substrate (Linhardt & Walter, 1965). Glucose-6-phosphatase (E.C. 3.1.3.9) was assayed in the presence of 4 mM-EDTA and 2 mM-KF (Harper, 1965; Hübscher & West, 1965). Na^{+}K^{+}-activated ATPase (E.C. 3.6.1.4) was determined by the method of Post & Sen (1967). The inorganic phosphate released was determined by the method of Fiske & SubbaRow (1925). Protein was determined according to Lowry et al. (1951).

Electron Microscopy. For transmission microscopy, membrane pellets were fixed on coverslips with 3% glutaraldehyde in .2 M-sodium cacodylate buffer (pH 7.4). After fixing overnight at 2°C, the specimens were washed and fixed secondarily with 1% osmium in a 200 mM-sym-collidine buffer (pH 7.4) for two h. After rinsing, the samples were dehydrated stepwise in 70, 95 and 100% alcohol. After treatment with propylene oxide and overnight infiltration with Epon 812/araldite 502, the sediments were embedded in the epon mixture. This was followed by polymerization at 60°C, then the coverslip was removed and the block was sectioned in a Sorvall MT-2

ultramicrotome. After staining with uranyl acetate and lead citrate,

the sections were examined with a JOEL 100C electron microscope.

For scanning microscopy, membrane sediment was spread thinly over

a coverslip and quickly fixed with 3% glutaraldehyde. In some cases the

specimen was fixed secondarily with osmium tetroxide. This was followed

by washing, dehydration, and immersion in amyl acetate or absolute acetone.

They were then critical point dried using liquid CO_2 to replace the liquid

solvent. After drying, the coverslips were mounted, coated with a thin

layer of palladium and gold and examined with a JOEL JSM-35 scanning

electron microscope.

Materials

Dextran 110 and Ficoll (MW ≈ 400,000) were purchased from Pharmacia

(Piscataway, N.J., U.S.A.) and α-N-tosyl-L-arginine(^{3}H)methyl ester from

Biochemical and Nuclear Corp. (Burbank, Ca., U.S.A.). Bradykinin, hip-

purylglycylglycine and sucrose (ultrapure) were from Schwarz-Mann (Orange-

burg, N.Y., U.S.A.). The kallikrein inhibitor aprotinin (Trasylol) was

obtained from Bayer AG, Prof. G. Haberland (Wuppertal-Elberfeld) and SBTI

from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). The synthetic

converting enzyme inhibitor SQ 20881 was obtained from Dr. Z. Horowitz

of Squibb, Inc. (Princeton, N.J., U.S.A.). All other reagents used were

of analytical grade.

Results

Microsomal Membranes. Using Procedure I we found that the relative

specific activities of marker enzymes of both ER and PM were elevated in

the washed microsomal fraction (Table 1). The recovery and relative

specific activity of kallikrein in the washed microsomes was also high

and similar to that reported by Nustad (1970). The extensive washing, which removes both adsorbed and intraluminal cytoplasmic contamination, removed approximately 60% of the protein of the microsomal pellet but none of the membrane markers or membrane kallikrein.

Fractions rich in either PM or in ER were prepared from washed microsomes of twenty-one rats in six experiments, using sedimentation in a discontinuous gradient of Dextran 110. The PM-enriched fraction, collected at the gradient interface of the Dextran 110 showed low glucose 6-phosphatase activity and high alkaline phosphatase and Na^+K^+-ATPase activities. The ER-enriched fraction which pelleted had high glucose 6-phosphatase activity but lower alkaline phosphatase and Na^+K^+-ATPase activities (Table 1). The specific activity of glucose 6-phosphatase in the ER-enriched fraction was nearly eight times that of the PM-enriched fraction while the specific activities of alkaline phosphatase and Na^+K^+-ATPase were one half and one third of those of the PM-enriched fraction.

Each fraction contained approximately 10% of the total kallikrein activity of the crude homogenate. The specific activity of the PM-enriched fraction in bioassay was five times that of the ER-enriched fraction and twenty-eight times that of the crude homogenate (Table 1). Preliminary experiments had shown that α-N-tosyl-L-arginine(^{3}H)methyl esterase activity in the homogenate was not due entirely to kallikrein. However, similar to bioassay of kallikrein, the specific activity of the esterase in the PM-enriched fraction was approximately three times that of the washed microsomal fraction and six times that of the ER-enriched fraction.

The kininase activities in the PM-enriched and ER-enriched fractions were similar. However, EDTA, a potent kininase inhibitor, was used in the

preparation of these membranes and even transient exposure to this inhibitor may affect the apparent distribution and activity of renal kininase.

This procedure demonstrated that renal microsomal kallikrein was more active in the PM-enriched fraction than in the ER-enriched fraction. Renal cortical tissue has a variety of cell types and plasma membranes of individual renal cell types are themselves heterogeneous (Bloom & Fawcett, 1968). Therefore, we isolated ER-enriched and PM-enriched fractions of renal cortex by a second procedure as well.

Microsomes were prepared seven times from pooled rat renal cortices using Procedure II. Microsomal membranes were subfractionated into PM-enriched and ER-enriched fractions by rate differential centrifugation using a linear continuous sucrose-Ficoll gradient as described in Methods. Figure 1 shows the relative specific distribution of the ER and PM marker enzymes glucose 6-phosphatase and alkaline phosphatase and of kallikrein, total kininase, angiotensin I-converting enzyme (kininase II) and angiotensinase. The distribution of the marker enzymes glucose 6-phosphatase (ER) and alkaline phosphatase (PM) demonstrates the separation of ER at the top of the gradient from PM containing brush border at the bottom. After pelleting, the structure of the membranes collected at the top and bottom of the gradient was examined by transmission electron microscopy. The top fractions contained both smooth and rough ER vesicles (Figure 2). The bottom of the gradient was enriched in PM and contained brush border membrane fragments. This separation of ER from PM is similar to that reported by Jakobsson (1974). Although other PM marker enzymes were not determined in the present study, the distribution of all PM-bound enzymes paralleled that of alkaline phosphatase according to Jakobsson (1974).

Figure 1 shows that the relative specific distribution of renal microsomal kallikrein can be resolved into two separate peaks parallelling both ER and PM fractions. Renal kininase, angiotensin I-converting enzyme (or kininase II) and angiotensinase (Figure 1) were concentrated in the fractions that contained the plasma membrane. The relative specific distribution of these three enzymes was nearly identical with that of the marker enzyme alkaline phosphatase. Unlike in Procedure I, no kininase, converting enzyme or angiotensinase inhibitors were used during the isolation of these membrane fractions.

Brush Border. Brush borders of the proximal tubules make up a significant fraction of the PM of renal cortex. We purified intact brush borders from the proximal tubule from twenty-seven rats in nine experiments as shown by marker enzymes and electron microscopy. Alkaline phosphatase was used as the marker enzyme of renal brush border and its relative specific activity in our purified brush-border fraction was nearly identical to that reported (Wilfong & Neville, 1970; Table 2). Although some contamination by ER was present, the relative specific activity of glucose 6-phosphatase remained near unity.

Electron microscopy confirmed that our preparation consisted of morphologically intact brush border (Figure 3). Transmission electron microscopy revealed thin sections of cytoplasmic fragments which represent the apical surface of the renal tubular epithelial cells. The brush borders of the cells are seen as a series of fingerlike projections measuring 0.15 microns in width and up to 1.5 microns in length. Part of the underlying cytoplasm with occasional vesicles and small amounts of ER remained attached. Scanning electron microscopy showed that the specimens

Figure 3

consisted of spherical or irregular masses of cytoplasm with fingerlike projections representing the brush border of the tubular cells.

The relative specific activity of kallikrein in the final brush border preparation was very low (0.2; Table 2). Unlike the marker enzyme, little or no kallikrein activity was associated with the brush border. The lack of kallikrein activity on the brush border cannot be attributed to loss of enzymic activity since the total recovery of kallikrein from all fractions was essentially 100% (not shown in Table 2). In contrast to kallikrein, kininase, angiotensin I-converting enzyme (kininase II) and angiotensinase were concentrated in the brush border. The relative specific activities of total kininase, converting enzyme and angiotensinase in the brush border were 7.1, 10.3 and 7.2 respectively. These values were similar to those of the marker enzyme in the brush border. The angiotensinase inactivated angiotensin II at pH 7.4 and was inhibited completely by 1 mM o-phenanthroline.

Tumors of Proximal Tubules. Homogenates of transplanted kidney tumors originating from the proximal tubules MK_2 and MK_3 were prepared and assayed as described in Methods. No detectible activity of kallikrein was found either in the whole homogenates or in the solubilized homogenates of these tumors. Renal tissue, of both control and host animals, stored and prepared in identical fashion, had normal levels of kallikrein. We took the results of these experiments as an additional indication that kallikrein may not be produced in normal proximal tubules.

Unlike renal kallikrein, the activity of kininase, angiotensinase and angiotensin I-converting enzyme (Hall et al., 1976) in the MK_2 tumor homogenate was similar to renal tissue. These data provide strong evidence

for the existence of kininase and angiotensinase in normal proximal tubules.

Discussion

It has been shown that a high speed sediment, the microsomal fraction of the homogenized kidney cortex contains enzymes that liberate or inactivate vasoactive peptides. Kininase, angiotensin I-converting enzyme (Erdös & Yang, 1966; Yang et al., 1971; Erdös, 1976), angiotensinase (Matsunaga et al., 1968), kallikrein (Nustad, 1970; Ward et al., 1975b), and renin (Wilson et al., 1976) are among them.

By using two procedures different both in methods of homogenization and fractionation, we separated renal cortical microsomes into ER- and PM-enriched fractions. Extensive washing procedures were employed to remove adsorbed and intraluminal contamination, ensuring that the presence of the enzymes in the PM- and ER-enriched fractions was not due to cytoplasmic contamination. The separation of ER- and PM-enriched fractions was confirmed by the use of marker enzymes and electron microscopy. Centrifugation in Dextran 110 demonstrated that kallikrein activity was present in both ER- and PM-enriched fractions, but the highest specific activity was associated with the PM fraction. Separation by rate differential centrifugation on sucrose-Ficoll gradients also demonstrated an association of kallikrein with both ER- and PM-enriched fractions. In this case, however, the specific activity of kallikrein was about equally distributed between both fractions. Different isolation procedures do not necessarily produce identical membrane subpopulations. Nevertheless, both procedures do support the concept that kallikrein is present on both ER and PM. However, a significant fraction of microsomal kallikrein may also

be associated with other membranes in the microsomal fraction such as the Golgi apparatus. Renal kallikrein may be synthesized on ER and subsequently reoriented to a surface localization for activation and release.

It has been suggested that urinary kallikrein is secreted at the level of the proximal tubule (Werle & Vogel, 1960; Nustad, 1970), but brush border membranes from proximal tubules have little or no kallikrein. In addition, renal tumor cells, ultrastructurally resembling cells of the pars recta of the proximal renal tubule (Hruban et al., 1973), contained no measurable kallikrein. This lack of activity was not due to the presence of any detectible endogenous inhibitor, uninhibited kininase or lack of some exchangeable cofactor.

The absence of kallikrein from brush border of the proximal tubules, from tumors originating from proximal tubules and from filtrate of proximal tubules (Scicli et al., 1975; Carretero & Scicli, 1976) all suggest that urinary kallikrein does not originate from the proximal tubules.

In contrast to kallikrein, renal kininase, angiotensin I-converting enzyme (kininase II) and angiotensinase are present in high concentration in the brush border of proximal tubule. Electron microscopy showed the huge surface of the brush border where the enzymes are present.

The observed relationship of these enzymes with the PM-enriched fraction but not with the ER-enriched fraction can be interpreted in two ways. Either membrane-bound kininase, converting enzyme and angiotensinase are present on plasma membranes in general (including brush border) or they are located on the brush border exclusively. The former

view appears most likely. Renal converting enzyme has also been found
at sites other than brush border (Hall et al., 1976). However, the data
do indicate that all three membrane-bound enzymes are localized on the
outside membrane of renal cells, particularly concentrated in the brush
border of proximal tubules. Such a localization may facilitate the
cleavage of kinins and angiotensins on the cell surface.

The angiotensinase described may be identical with the aminopeptidase
angiotensinase A. Similar to renal angiotensinase A (Erdös, 1971), it
is a membrane bound enzyme, active at a neutral pH and inhibited by o-
phenanthroline. High concentration of an aminopeptidase was also found
in the brush border by Quirk & Robinson (1972).

If the production of kinin and angiotensin in the kidney affects
renal function, the kidney must be capable of inactivating such peptides
introduced into the kidney from extrarenal sources. When a kinin
(Abe,. 1965; Carone et al., 1975) or angiotensin II (Vane, 1969; Pullman
et al., 1975) is infused into the renal circulation, these peptides are
nearly completely inactivated. Little or no intact kinin infused into
the renal artery reaches the venous effluent (Nasjletti et al., 1975)
or is excreted into urine which, however, contains kinins (Werle & Erdös,
1954; Miwa et al., 1968; 1969). The low molecular weight of these peptides
makes it likely that any plasma kinin or angiotensin II which escapes
inactivation in the circulation is filtered at the glomerulus. Function-
ally oriented enzymes on the huge surface of the brush border of the
proximal tubules could then inactivate both peptides. We may speculate
that kallikrein or even renin bound to membranes (Wilson et al., 1976),
located distal to the proximal tubules, can release kinins or angiotensins
that may alter ion transport and liberate prostaglandins.

Acknowledgements

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Table 1

Distribution of Enzyme Activity in Membrane Fractions

	Kallikrein		^{3}H α-N-Tosyl-1-l-arginine methyl ester hydrolysis		Glucose 6-phosphatase activity	
	Sp. activity (ng of bradykinin equiv. released/min per mg of protein)	Relative sp. activity	Sp. activity (amount of the compound hydrolysed/min per mg of protein) (as c.p.m.)	Relative sp. activity	Sp. activity (µmole of substrate hydrolysed/min per mg of protein)	Relative sp. activity
Homogenate	1.0 ± 0.2	1	1290 ± 210	1	0.05 ± 0.001	1
Washed microsomes	9.5 ± 2.1	9.5			0.33 ± 0.03	6.5
Plasma membrane fraction	28 ± 5.1	28	3100 ± 350	2.4	0.06 ± 0.01	1.1
Endoplasmic-reticulum fraction	5.6 ± 1.1	5.6	520 ± 130	0.4	0.43 ± 0.09	8.5
No. of experiments	4	2		5		5

The preparation of membrane fractions and the assay of enzymes were as described under "Methods". All values are in the fraction)/(mean specific activity in the homogenate).

(the specific activity in the fraction)/(mean specific activity in the homogenate).

Isolated from Rat Kidney Cortex

	Alkaline phosphatase		Na$^+$ K$^+$ ATPase	
	Sp. activity (µmole of substrate hydrolysed/min per mg of protein)	Relative sp. activity	Sp. activity (µmole of substrate hydrolysed/min per mg of protein)	Relative sp. activity
	0.10 + 0.004	1		
	0.52 + 0.11	5.3	0.14 + 0.12	1
	0.64 + 0.13	6.5	0.38 + 0.14	2.7
	0.35 + 0.09	3.6	0.12 + 0.05	0.8
	3		3	

means + S.E.M. Relative specific activity is (mean specific activity

Table 2

Enzyme Activity of the Isolated Brush Border Preparation

of Rat Kidney Cortex

	Specific Activity	Relative Specific Activity
Alkaline Phosphatase	1.20 ± 0.22	15.0
Kallikrein	0.4 ± 0.20	0.2
Kininase *	2.3 ± 0.15	7.1
Angiotensin I converting enzyme (Kininase II)†	1.2 ± 0.15	10.3
Angiotensinase *	0.6 ± 0.10	7.2
Glucose 6-Phosphatase	0.05 ± 0.004	1.0

Brush border membranes were prepared as described by Wilfong & Neville (1970). All values given as means ± S.E.M. of five experiments. Relative specific activity is (mean specific activity in the brush border fraction) / (mean specific activity in the homogenate). Specific activities are defined in Table 1.

* µg of bradykinin (angiotensin II) hydrolysed/min per mg of protein

† nmole of hippurylglycylglycine hydrolysed/min per mg of protein

Legends

Figure 1

Distribution of glucose 6-phosphatase (O), alkaline phosphatase (Δ), kininase (◐), angiotensin I-converting enzyme (▯), kallikrein (▲), and angiotensinase (◪) after rate differential centrifugation of the microsomal fraction of homogenized cortex of the rat kidney in a sucrose-Ficoll gradient. Relative specific activity is (mean specific activity of fraction) / (mean specific activity of microsomal fraction).

Figure 2

Transmission electron micrographs of subfractions of renal cortex after rate differential centrifugation in a sucrose-Ficoll gradient. See "Methods" and Figure 1. Subfractions 1-4 (top), subfractions 9-12 (bottom). Scale line represents 0.1 micron.

Figure 3

Transmission (top) and scanning (bottom) electron micrographs of brush border prepared as described under "Methods". Scale line represents one micron.

RELATIVE SPECIFIC ACTIVITY

FRACTION NUMBER
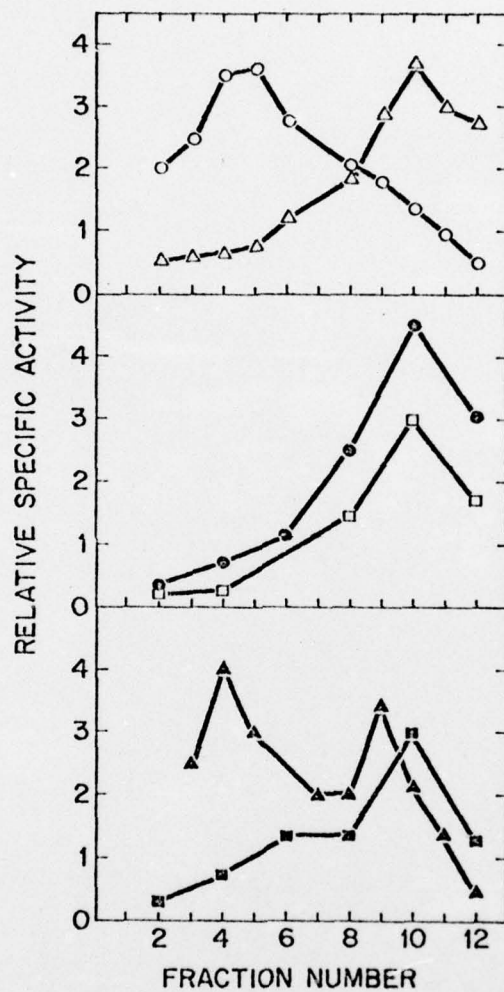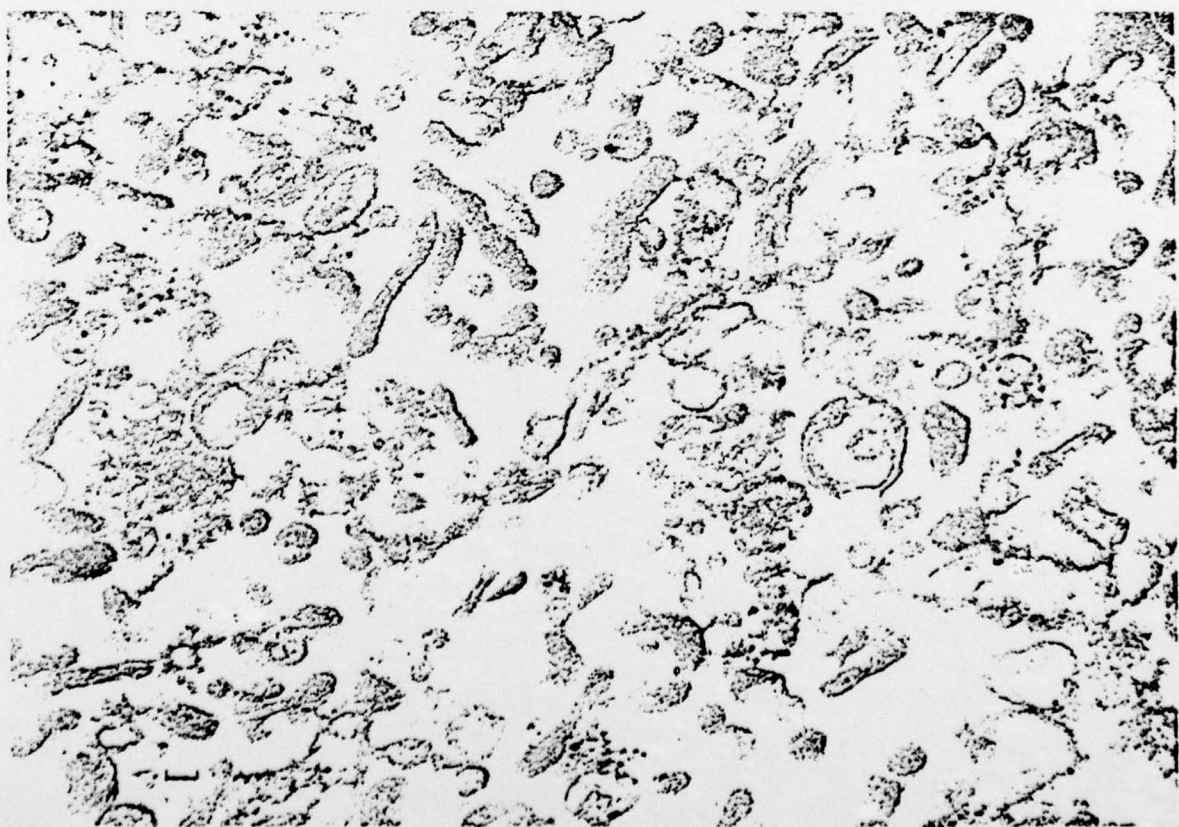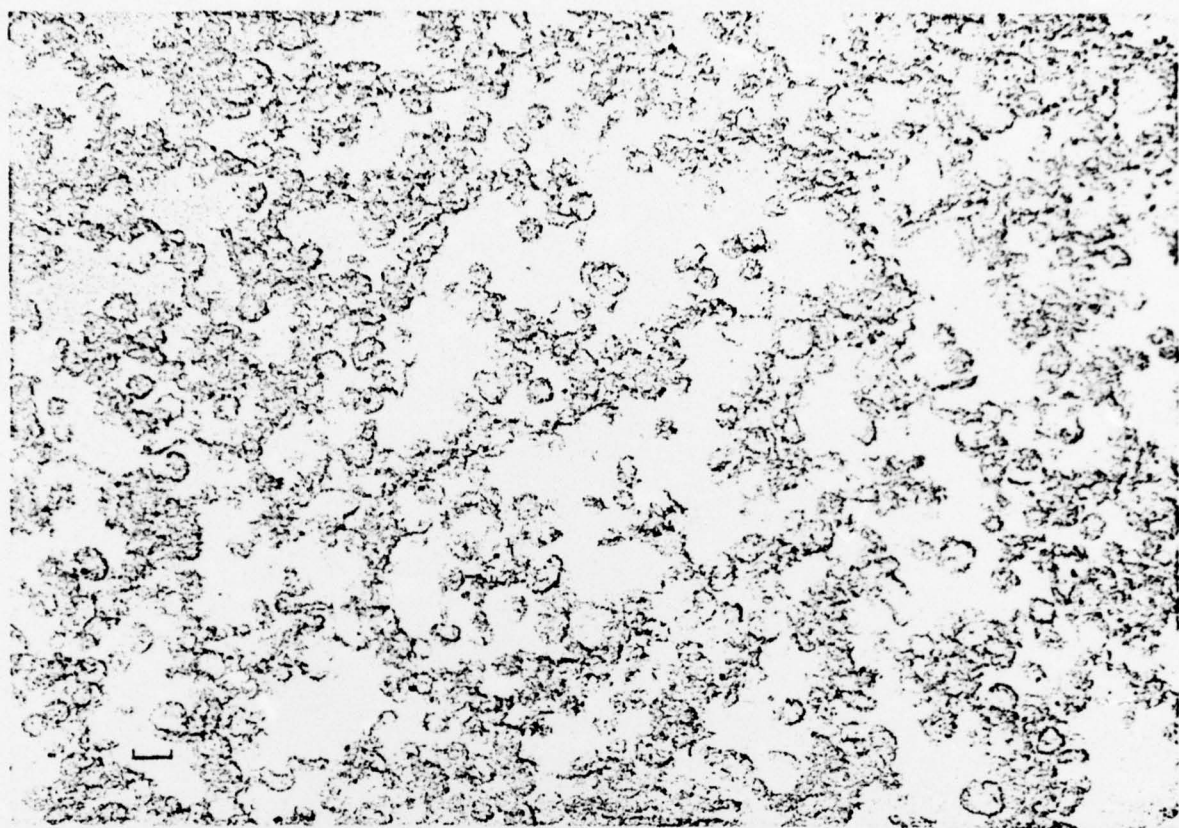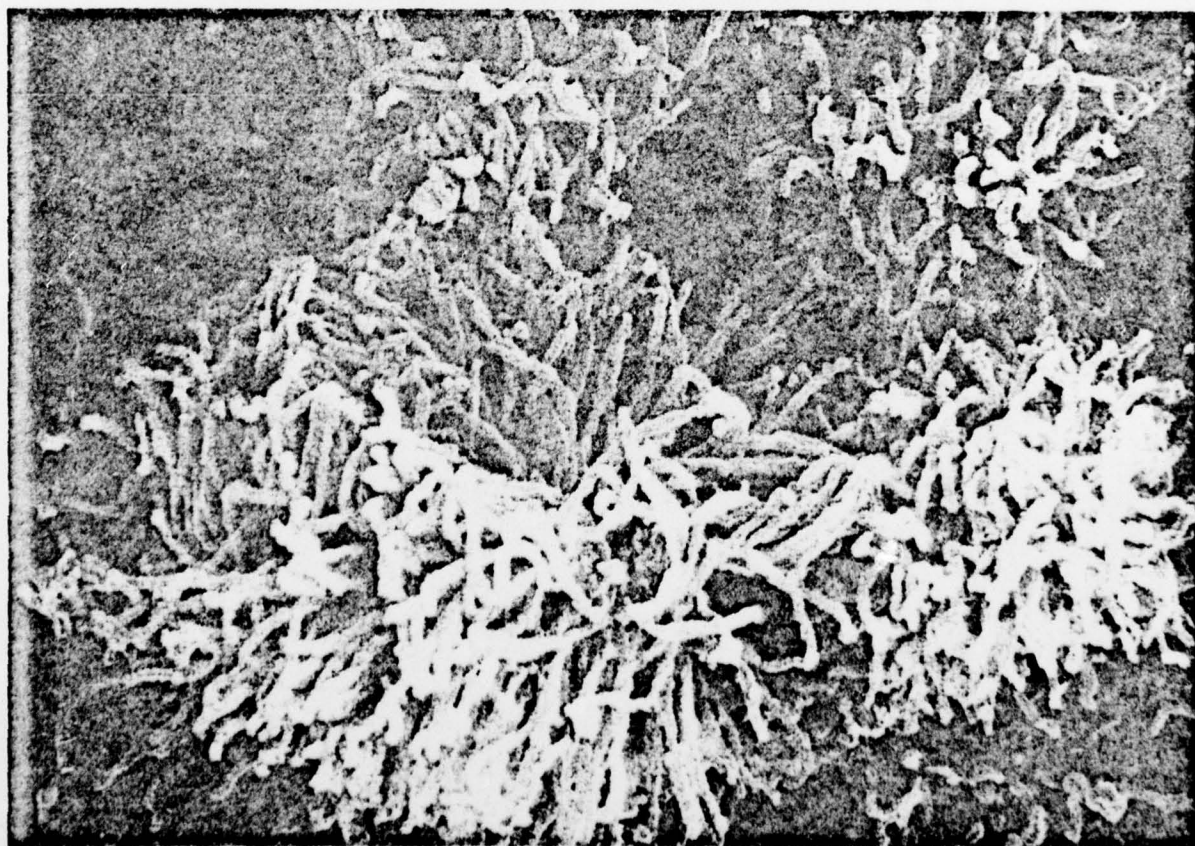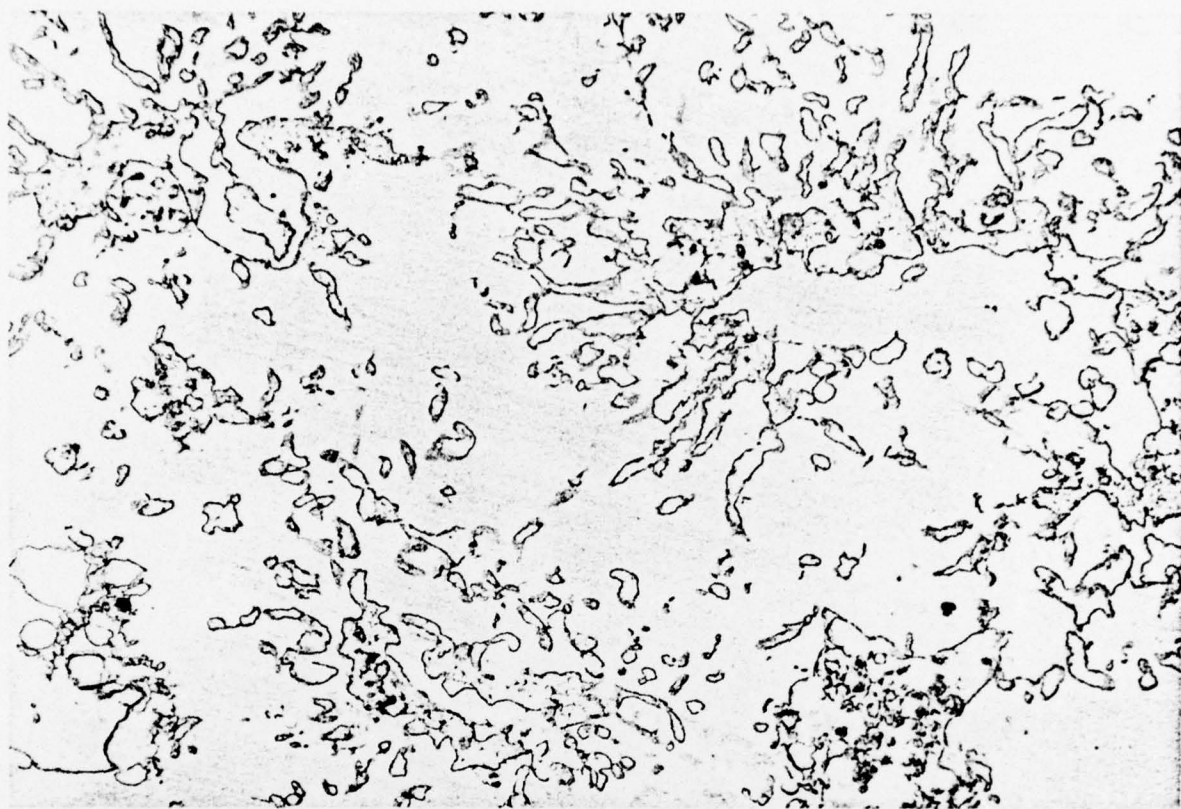
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